

A biosystematic evaluation of the relationship between three allopatric shrubby species of *Vernonia* SCHREB. (Asteraceae) in Nigeria

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Abstract

Biometric data and analyses of morphological attributes, reproductive biology and cytological traits of three allopatric shrubby species, *Vernonia tenoreana* OLIV., *V. stenostegia* (STAPF) HUTCH. & DALZ. and *V. kotschyana* SCH. BIP., are presented. The data were used to highlight the similarities and differences between the species with intent to enhance a biosystematic delimitation of the taxa.

Further details for the taxa delimitation are provided. There is evidence of character convergence as well as considerable degree of parallelism among the species. Morphological modifications are considered of adaptive significance and related to the environment of each species. They are, however, not viewed as sufficient for a change in nomenclature of the studied taxa or their generic relocation.

Introduction

HUTCHINSON & DALZIEL (1963) provided a taxonomic basis for the identification of *V. tenoreana* OLIV., *V. stenostegia* (STAPF) HUTCH. & DALZ. and *V. kotschyana* SCH. BIP. as separate species of *Vernonia* found in West Africa. However, a cursory collector of the materials from their natural habitats in Nigeria is most likely to misidentify them and hence group them as one and the same because of some obvious morphological similarities. AYODELE (1999) reported as a possible explanation that the delimitation might be influenced by some geographic habitat differences.

One tendency of a large size genus as *Vernonia* is the considerable variation among the species just as there is a considerable degree of convergence of attributes (JONES 1979). The tremendous diversity that has evolved in the genus *Vernonia* is demonstrated by the wide ecological preferences, the variable growth forms and the morphological traits that readily adapt the species to their accredited habitats (JONES 1979, AYODELE 2000).

A number of reports have highlighted the difficulty in the delimitation of taxonomic units in *Vernonia* (FAUST 1972, JONES 1979, ISAWUMI 1985). A need for a better understanding of the morphological variation in the group was long ago suggested (SMITH 1971). Commenting on the West African *Vernonia*, SMITH (1971) opinioned that the species were not clearly delimited, their delimitation having been based upon relatively few and poorly understood characters. For this reason, he in fact also declared the traditional sections of *Vernonia* as clearly artificial.

ISAWUMI (1985) noted the similarity between many West African *Vernonia* species and has since been consistent about this opinion (ISAWUMI 1993, 1995a & b), but allowed for a large number of taxa being transferred to another genus, *Baccharoides* (ISAWUMI et al. 1996). ISAWUMI (1995b) also reported that workers on the classification of *Vernonia* are not in total agreement as to the limits of the sections. These observations call for more information on parameters for species delimitation in the West African *Vernonia* group.

A better scientific understanding of species and their lineage is one major goal of biosystematics. This makes possible the comparative study of attributes, which can be interpreted in the light of evolutionary and genetic theory (ROSS 1974). Three attributes namely: the widespread distribution, the morphological variability and the ecological versatility of *Vernonia*, recommend the genus as highly suitable for biosystematic study (FAUST 1972, JONES 1979, AYODELE 2000).

The objective of this paper is to document the observed biometric variations in morphological, reproductive and cytological attributes within and among three shrubby species of *Vernonia* namely: *V. tenoreana*, *V. stenostegia*, and *V. kotschyana*. This is with a view to highlight any similarities and differences in morphological traits, reproductive biology and cytological attributes, that are germane for a biosystematic delimitation of the taxa.

Materials and Methods

Location Survey of the *Vernonia* Plants

Plant materials for investigations were obtained from field trips to different locations of high populations of the taxa as described in Table 1 for the various data collected.

Studies on the Morphological Characters

Observed morphological traits were measured and recorded from randomly sampled specimens at their natural habitats. These traits include plant height at flowering, and leaf, capitulum and fruit measurements by methods described by FAUST (1972) and modified by AYODELE (1997).

Studies on Reproductive Biology

The gross floral morphology and reproductive capacity of each species were studied through: plant branching traits, arrangement and number of capitula per branch or branchlet, number of florets per capitulum, morphological forms of phyllaries, flower maturation time, pollen morphology, fruit morphology, fruit production rate and fruit viability.

Cytological Studies

Karyomorphological studies were carried out on the species. Root tip cells were obtained from seedlings raised in the laboratory. Karyotype data based on average measurements of the chromosomes of 5–8 well-spaced metaphase plates for each species, were analysed and compared. Data included chromosome morphology i.e. absolute size, position of centromere (expressed as long arm/short arm ratio) and symmetry or asymmetry in arm length. Idiograms for each taxon were prepared, with all chromosomes arranged in order of decreasing length and assigned numbers.

Data Analysis

Means, standard deviations and ranges of all measurements taken, were determined for each species. The coefficients of variation (CV %) for the leaf, floral and fruit measurements, were computed to compare the variations in the attributes within each species. Statistical analysis of the data was done, employing analysis of variance and Duncan's Multiple Range Test.

All significant differences in the compared parameters measured were made by a -posteriori comparison (SOKAL & ROHLF 1969). Scatter diagrams of some morphological and reproductive attributes were constructed by means of the Genstat 5 Release 3.22 high-resolution graphics to highlight the relationships among the taxa.

Results

Morphological Variations Within and Between Accessions of *V. tenoreana*, *V. stenostegia* and *V. kotschyana*

Vernonia tenoreana, *V. stenostegia* and *V. kotschyana* are consistently erect shrubs in all their habitats (Tables 1 & 2). *Vernonia stenostegia* plants were heavily branched from the lower parts of the main stem. The stem and branches were very leafy during the vegetative growth phase. The branches were usually leafless at the lower parts during the peak of flowering of adult plants. In contrast, much of the broad leaves of *V. tenoreana* were retained even during the moisture stress period of the dry season. *Vernonia kotschyana* appears to be intermediate between these two extremes.

Variations in leaf measurements within and between taxa were quite obvious (Fig. 1a). There was no conspicuous difference in leaf shape of the three species (Table 5). The scatter diagram on leaf measurements (Fig. 2a) indicates that the three taxa belong to separate groups of leaf size. There is an overlap between *V. stenostegia* and *V. kotschyana*, both of which were distinct from *V. tenoreana* (Tables 2 & 3). Other records of morphological attributes of value in species delimitation can be found in Tables 2, 3 and 5.

Reproductive Attributes of *V. tenoreana*, *V. stenostegia* and *V. kotschyana*

Floral measurements show significant differences ($P = 0.05\%$) between *V. stenostegia* on one hand, and the other two species (Table 3, Fig. 2). The infraspecific variation in capitulum diameter was conspicuously smaller in both *V. tenoreana* and *V. kotschyana* (Fig. 1b). These two species also belong to the same capitulum-size group with heads larger than those of *V. stenostegia* (Fig. 2b). The florets (the corolla length in particular) of *V. stenostegia* were conspicuously smaller than those of the other two species. This was consistent for all accessions of the taxa from different habitats. There is no significant difference among the number of florets contained in the capitula of the three species (Tables 3 & 5). Other floral characters of value in delimitation can be found in Table 2 and Fig. 3. The differences in the phyllaries of the taxa are quite distinct with regard to shape, texture and number per capitulum (Table 2).

Of the three species, *V. stenostegia* produced more capitulum-clusters in which there were a higher number of capitula. The mode of inflorescence branching clearly distinguishes the three species (Table 2). The differences in pollen and fruit characters were significant among the species (Tables 3, 4 & 5). The pappus bristles were different in length while their surface micro-characters were different in the number of multicellular spine-like projections at the top, middle and lower portions respectively.

Cytological Variations Among the Taxa

The three species have a diploid chromosome number of $2n = 20$ ($x = 10$). The karyotype of their chromosomes manifests varying morphology, the details of which are contained in Table 6. There were marked differences in the length of chromosomes and the absolute length of chromosome complements. *Vernonia kotschyana* had the shortest chromosome complements (Table 6 & Fig. 4). The CTL values were also highly variable among the species ($CV = 36.26\%$), the value being lowest in *V. kotschyana* (2.08 μm). The relative sizes of the chromosome complements (Fig. 4) in *V. tenoreana* and *V. stenostegia* appear rather more homogeneous than those in *V. kotschyana*.

The centromere positions in the three species were variable and heterogeneous (Fig. 4). Striking shifts of centromere were observed in chromosome pairs 6 and 10 of *V. tenoreana* and chromosome 7 in *V. kotschyana* (Fig. 5). The chromosomes of the

species clearly display a considerable degree of asymmetry in arm ratio values (Table 6, Fig. 5). The arm ratio values are high especially in those chromosomes with striking shifts of centromere positions. Generally, there is variation in the extent of heterogeneity in centromere positions and the number of chromosomes showing the heterogeneity.

Discussion

The leaf measurements (Fig. 2a and Tables 1 & 3) reveal the morphological association between *V. stenostegia* and *V. kotschyana*. However, *V. tenoreana*, the species with broader leaf, maintains its identity of a plant form growing in the more humid environment of the derived savanna in the rain forest zone. Of the three taxa, *V. stenostegia* has the narrowest ecological amplitude with the apparent restriction of its natural populations to the Guinea savanna zone only (AYODELE 2000). The other two species especially *V. tenoreana* are well distributed further from the savanna grassland into the rainforest zone.

The observed relatively narrower leaf especially of *V. stenostegia* is therefore, a manifestation of the property of plants growing in a moisture stress habitat. Usually the dry and exposed habitats have the highest proportion of plants with small leaves (STERN & ROCHE 1974, SANFORD & ISICHEI 1981). The convergence of the relatively small size leaf attribute in *V. stenostegia* and *V. kotschyana* (Fig. 2a) is thus a demonstration of the need for smaller size broad leaf shape, a moisture conservation strategy essential in a dry habitat where they are found. The high amplitude of intra-specific variation in leaf size of *V. tenoreana* collected across these vegetation zones corroborates this (Fig. 1a).

Certain floral attributes are very useful tools in plant taxonomy because of their consistency despite environmental factors (BURTT 1977). The capitula of *V. tenoreana* and *V. kotschyana* that are also morphologically associated (Fig. 2b) were grouped in the large size class (AYODELE 1995). Their sizes are apparently unaffected by changes in environmental fortunes. This is particularly true of *V. tenoreana* collected across varying vegetation habitats.

Similarly consistent for each taxon are the differences in their phyllaries with regard to shape, texture and number per capitulum (Table 2). This attribute clearly separates the taxa. So does also the corolla size especially in *V. stenostegia* (Fig. 3). The texture of the phyllaries and leaves in *V. stenostegia* is indicative of its xeric habitat. Other reproductive factors contributive to a clear delimitation of the taxa include the mode of inflorescence branching, the significant differences in pollen, and fruit features (Fig. 2c; Tables 2, 3, 4 & 5).

AYODELE (1995) reported the presence of multicellular spine-like projections on the otherwise smooth-looking pappus hairs on cypselas of 16 species of *Vernonia*. From his observation of inter-species variation in the quantity of these 'spines' at the top, middle and lower portions of a pappus hair respectively, he suggested the use of this character for delimitation of taxa among growth forms and for species within a growth form. The taxa under study can be delimited by the surface micro-characters of their pappus (Plate 1). This is in addition to the fact that they differ significantly in pappus length (Table 5).

The distinctive differences in cytological attributes among the taxa (Table 6, Figs.4 & 5) suggest the possibility of a phenetic delimitation of the taxa confirmable with modern molecular genetics. A prominent problem in the taxonomy of *Vernonia* is the diversity of ecological preferences and the associated convergence of morphological attributes. This is made more complex by the observed adaption strategies to differing environments that have brought about the diversity and ecological versatility.

Morphological attributes constitute the orthodox tool of the taxonomist because of their diagnostic value in taxonomic evaluation. It must however be borne in mind that the morphology of an organism is not simply an accident. It represents the result of a long evolution of successive adaptation of living things to their environment (BALDAUF 1965). JONES (1973) observed that morphological discontinuities in *Vernonia* are representative of the genetic differences caused by adaptation to differential climatic and edaphic conditions. It is in this purview that a holistic biosystematic approach is being suggested for analytical consideration of plant taxonomy adjustments or generic relocations. This will prevent a chaotic reclassification of plants upon every circumstantial morphological evidence, devoid of other equally important reasons for the observed similarities or dissimilarities in traits.

This call is necessary in view of suggestions for changes and corrections to names of Nigerian plants, which unite *V. tenoreana*, *V. stenostegia* and *V. kotschyana* under the name *Vernonia adoensis* (LOWE & SOLADOYE 1990). In a review of some of such united or renamed species, LOWE & SOLADOYE (1990) quoted floras and other taxonomic publications as their sources of information on the change of names. ISAWUMI (1995a & 1995b) also proposed a total relocation of the homonym and its constituent taxa to a 'resurrected' genus *Baccharoides*, followed later by numerous more transfers (ISAWUMI et al. 1996). This proposal was based on some discovered morphological attributes and in particular, floral micro-characters (ROBINSON 1990, ISAWUMI 1993, 1995a & b, ISAWUMI et al. 1996). Such re-classifications may result in the loss of appreciation of adaptation strategies developed over time by plant species to promote an individualistic identity.

Different workers display different emphasis in plant classification. Classification primarily, should be an information retrieval system that may also almost as a subsidiary feature, indicate evolutionary relationship. A correctly determined evolutionary relationship will produce a useful information retrieval system. It may therefore, be necessary to always investigate the genetic basis and adaptive significance of morphological features before they are propounded as sole reason for any form of nomenclature adjustments or re-classification of plants.

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Table 1. Population, location & collection data of *V. tenoreana*, *V. stenostegia* & *V. kotschyana*

Name & accession number of species	Location of collection	Description of location
<i>Vernonia tenoreana</i> OLIV. msa 040, 041, 043, 048 & 051	Routes: Ede/Awo, Osogbo/Ede, Osogbo/Ilesa, Olupona/Iwo, Oyo/Awe, Ilorin/Igbeti; Township: Ogbomoso/Igbeti	Guinea & Derived savanna grassland with sandy loam soil; uncultivated burnt forest vegetation, abandoned plots, and weedy roadsides
<i>Vernonia stenostegia</i> (STAFF) HUTCH. & DALZ. msa 061, 069, 070 & 076	Routes: Gimi/Jos, Jos/Akwanga, Jos/Kuru; Township: NIVR - Vom, & Jos.	Guinea & Plateau open rolling grassland with altitude cooling effect; fallow plot in sandy, sometimes rocky woody valleys in shallow soils
<i>Vernonia kotschyana</i> SCH. Bip. msa 071, 081 & 082	Routes: Jos/Bauchi; Township: Bayero University Kano & ABU premises Zaria	Guinea savanna grassland with sandy/gravelly soil; abandoned plots and weedy roadsides

Table 2. Morphological features of stem, leaf, flower and fruit of *V. tenoreana*, *V. stenostegia* & *V. kotschyana*

Morphological features					
Plant stem and leaf features		Flower head (capitulum) features			Fruit features
Species/stem & growth habit	Leaf shape (tip & base)	Phyllaries	Corolla (form & colour)	Type of inflorescence branching. Flowering & fruiting season	Cypsela shape, pubescence & ripe pappus colour
<i>V. tenoreana</i> Erect tall-growing (81-185 cm) woody shrub with few branches (3-5) & tuberous rootstock; leafy stem branches terminating in a few but large flower heads	Ovate Acute-rounded, acute	Herbaceous & polyseriate; inner layer lanceolate and interlocking	Fluted Creamy white	Racemose arrangement May to December	Terete with groove on fruit surface Glabrous Buff
<i>V. stenostegia</i> Erect woody shrub (50-185 cm high), slender stem & many branches; stem leafless below but terminating in many heads at fruiting peaks	Obovate Acute, cuneate	Acicular, uniseriate, tomentose, woolly surface	Fluted Pale bluish to purple	Racemose arrangement of cymes September to March	Terete Pubescent (sericeous) Light brown
<i>V. kotschyana</i> Erect short woody shrub (38-90 cm at flowering), sparsely branched with branches terminating in large corymbose heads	Obovate to oblong Acute, acute	Herbaceous & polyseriate; outer layer more or less curled bracts	Fluted Light purple tubular florets, creamy white at full bloom	Racemose arrangement of umbels September to December	Terete Scabrid Buff

Table 3. Measurements of morphological characters of *V. tenoreana*, *V. stenostegia* & *V. kotschyana*

Leaf				Floral (Capitulum)				Fruit (Cypsela)									
Length (cm)		Width (cm)		L/W ratio		Length (cm)		Diameter (cm)		# Florets/ capitulum		Length (cm)		Width (cm)		Pappus length (mm)	
\bar{x}	Range	\bar{x}	Range	\bar{x}	Range	\bar{x}	Range	\bar{x}	Range	\bar{x}	Range	\bar{x}	Range	\bar{x}	Range	\bar{x}	Range
<i>V. t.</i>	20.3 – 26.2	10.6	7.8 – 13.4	2.63	2.37 – 3.09	3.42	3.3 – 3.7	3.46	3.1 – 3.7	135.4	111 – 169	5.20	4.1 – 6.0	1.46	1.2 – 1.9	12.75	12.0 – 13.9
<i>V. s.</i>	11.9 – 13.4	5.0	4.1 – 6.2	2.72	2.16 – 3.15	2.64	2.4 – 3.0	2.41	1.7 – 2.8	131.6	112 – 160	3.32	2.7 – 4.4	1.27	1.0 – 1.6	7.82	6.8 – 8.7
<i>V. k.</i>	15.2 – 17.3	6.53	5.4 – 7.4	2.70	2.31 – 3.02	3.46	2.7 – 4.1	3.14	2.8 – 3.3	146.2	131 – 168	5.53	4.2 – 6.6	1.8	1.6 – 2.1	12.18	10.8 – 13.2

Key: *V. t.* = *Vernonia tenoreana*; *V. s.* & *V. stenostegia*; *V. k.* = *V. kotschyana*Table 4. Summary data on some reproductive attributes of *V. tenoreana*, *V. stenostegia* & *V. kotschyana*

Species	Pollen				Flowering		Fruiting		Cumulative seed germination % (days after sowing)		
	Size (μ) \bar{x} (\pm Sd)	CV %*	Quantity index (\pm & Sd)	% Fertility	Initiation range **	Duration range ***	Fruit-set %	Wt of 10 fruits (mg) (\bar{x} & Sd)	0-7 days	7-14 days	14-21 days
<i>V. tenoreana</i>	69.8 \pm 7.05	10.1	35.4 \pm 11.8	98.83	155–160	120–210	92.9	59.16 \pm 12.99	10.8	63.0	71.0
<i>V. stenostegia</i>	61.4 \pm 4.02	6.5	23.5 \pm 12.8	99.44	130–135	110–180	79.6	17.80 \pm 1.43	6.0	16.0	17.6
<i>V. kotschyana</i>	59.8 \pm 8.61	14.4	9.1 \pm 2.96	100.00	78–140	100–180	30.9	70.8 \pm 3.11	13.4	33.3	89.0

* Intra-species pollen size coefficient of variation

** First appearance of flower buds in days after seed sowing

*** Period of continuous flowering in a season

Table 5. Summary ranking of scored values of morphological & reproductive attributes

		Duncan's Multiple Test Ranking & Score index		
S/N	Morphological/Reproductive attributes	<i>V. tenoreana</i>	<i>V. stenostegia</i>	<i>V. kotschyana</i>
1.	Leaf length (cm)	8a	5c	6b
2.	Leaf width (cm)	7a	5c	6b
3.	Leaf shape index *	6a	6a	6a
4.	Capitulum length (cm)	9a	8b	9a
5.	Capitulum diameter (cm)	9a	7c	8b
6.	# Florets/capitulum	7b	7b	8a
7.	Fruit length (cm)	8b	5c	9a
8.	Fruit width (cm)	8b	7c	9a
9.	Pappus length (cm)	9a	7c	8b
10.	# Branches/plant	5b	8a	4c
11.	# Terminal capitula clusters	4b	7a	1c
12.	# Capitula/terminal clusters *	7a	7a	7a
13.	Estimated seed production/plant	6b	8a	6b
14.	Plant height at flowering (cm)	5a	5a	3c
15.	Pollen size	9a	8b	8b
16.	Pollen quantity index	9a	7b	5c
17.	Pollen viability *	9a	9a	9a

Notes:

1. Species with the same letters have values not significantly different at 5 % level for the attribute.
2. * Major attribute convergence features among the taxa.

Table 6. Summary data on chromosome morphology of the three taxa

Species (Chromosome number $2n = 20$)	n	Number of chromosomes in each group			Size of chromosome complement		
		M	nm	nsm (-)	CTL * (μ)	TF % **	Coefficient of variation (CV %) in chromosome length
<i>V. tenoreana</i>	10	5	2	3	3.30	43.64	12.12
<i>V. stenostegia</i>	10	6	2	2	2.70	45.19	12.71
<i>V. kotschyana</i>	10	1	6	3	2.08	70.67	15.83

Notes:

1. * Cumulative total length or the index of the volume of all chromosomes in set (after GRANT 1975).
2. ** Total Chromosome Form Percentage calculated as:

$$\frac{\text{Total length of all short arms}}{\text{Total length of chromosome complement}} \times 100$$

Fig. 1a. Intraspecies variation in leaf measurements of the taxa

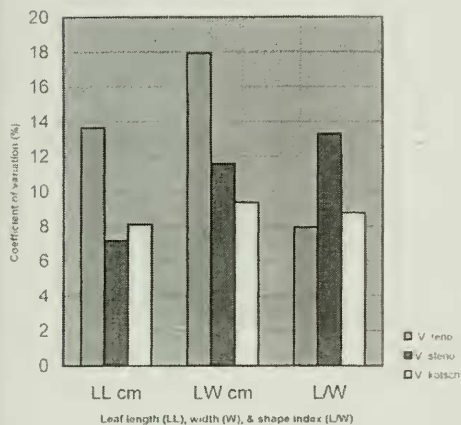


Fig. 1b. Intraspecies variation in capitulum measurements of the taxa

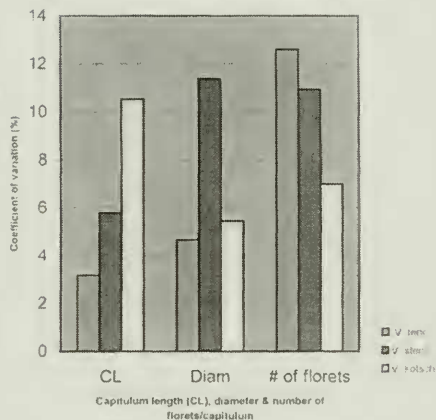


Fig. 1c. Intraspecies variation in fruit measurements of the taxa

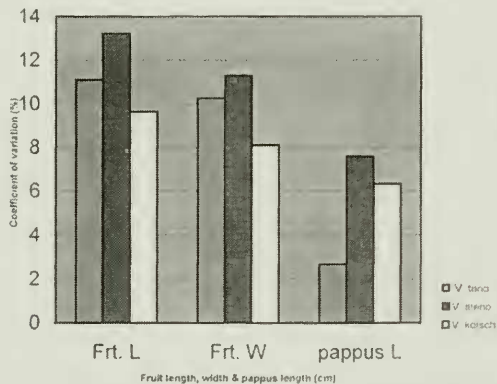


Fig. 1. Intraspecies variation in leaf, capitulum and fruit measurements

Fig. 2a. Scatter Diagram of Leaf Measurements

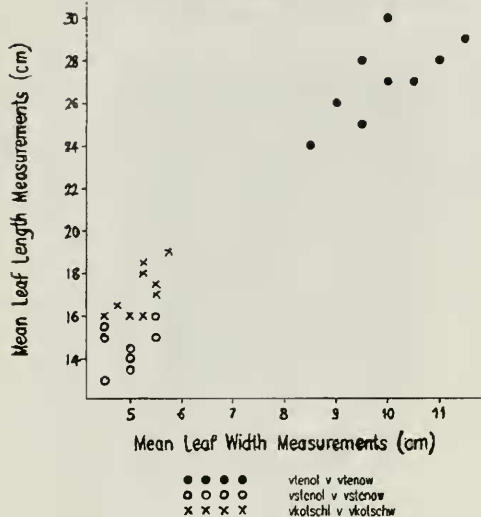


Fig. 2b. Capitulum Measurement of the Taxa

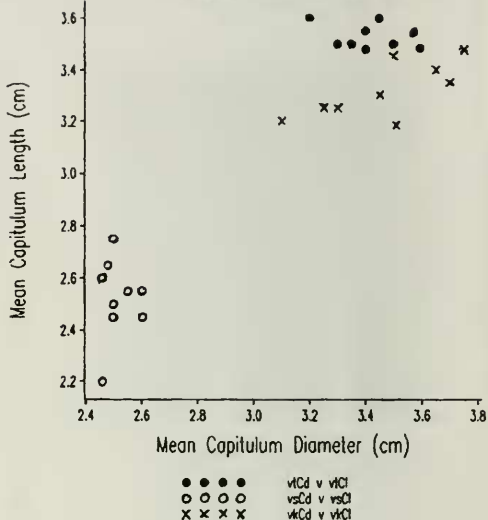


Fig. 2c. Scatter Diagram of Fruit Measurements (mm)

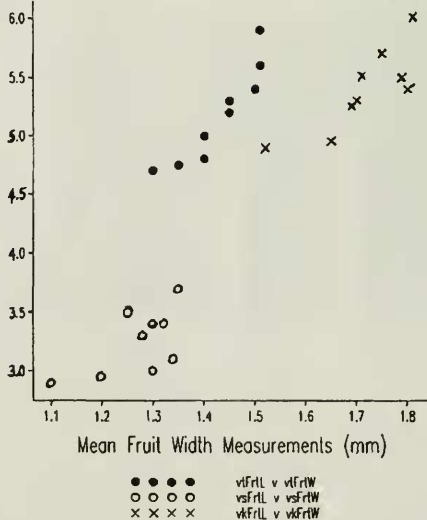


Fig. 2a. Scatter diagram of leaf measurements

Fig. 2b. Capitulum measurement of the taxa

Fig. 2c. Scatter diagram of fruit measurements (mm)

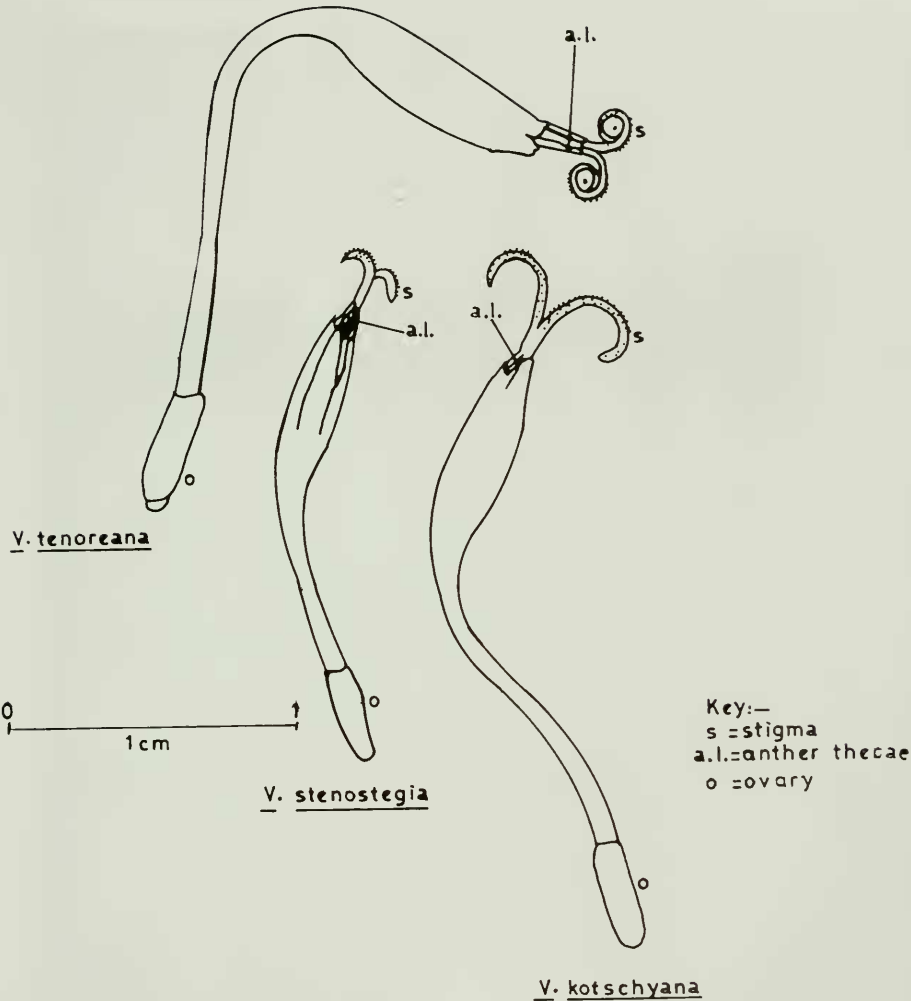


Fig. 3. Drawing of shapes and types of florets of the taxa

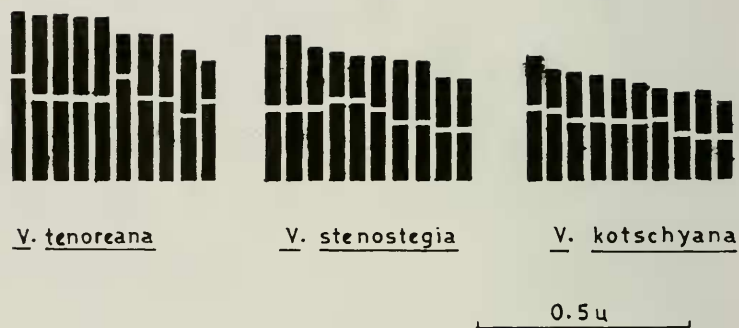


Fig. 4. Idiograms of *V. tenoreana*, *V. stenostegia* and *V. kotschyana*

Fig. 5. Arm ratios r (l/s) of the component chromosomes of the taxa

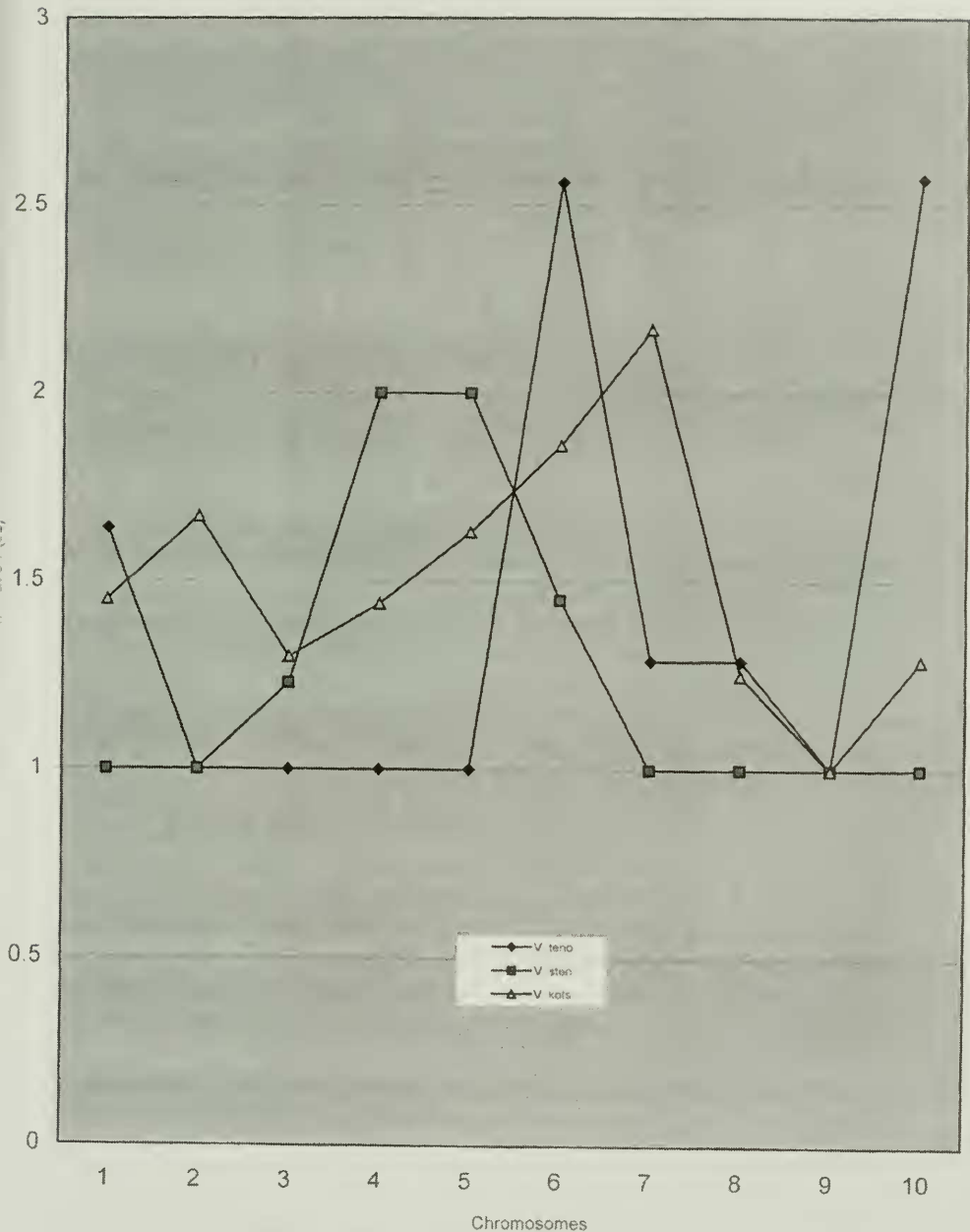


Fig. 5. Arm ratios r (l/s) of the component chromosomes of the taxa